

FIG 1

095644-04300

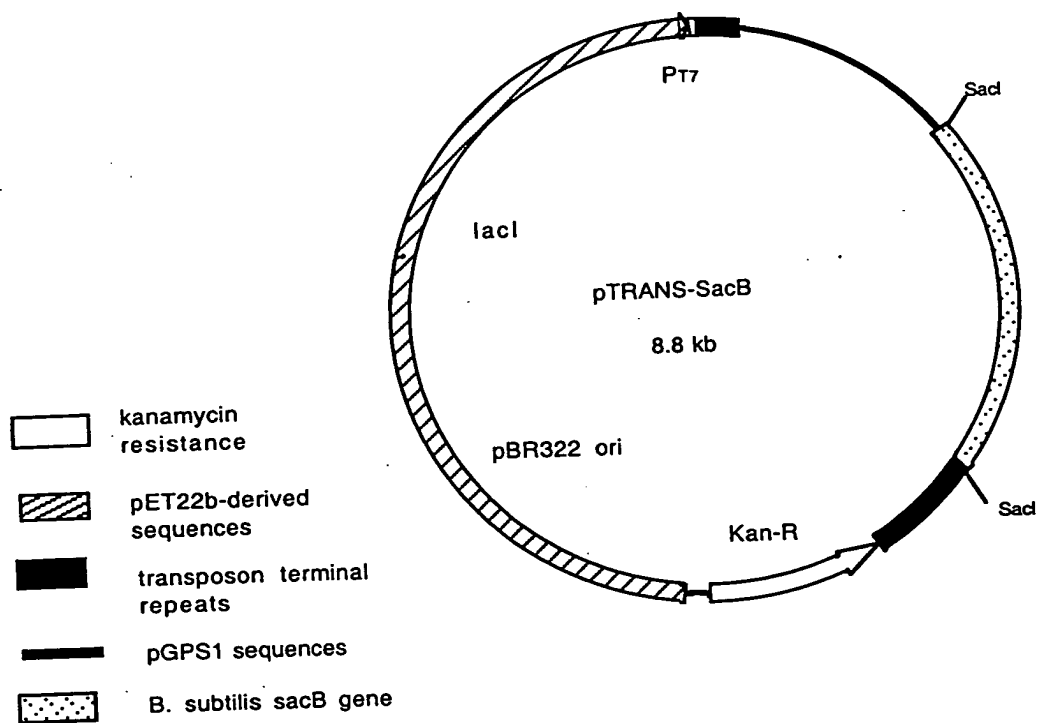


FIG 2

- kanamycin
resistance
- pET22b-derived
sequences
- transposon terminal
repeats
- pGPS1 sequences

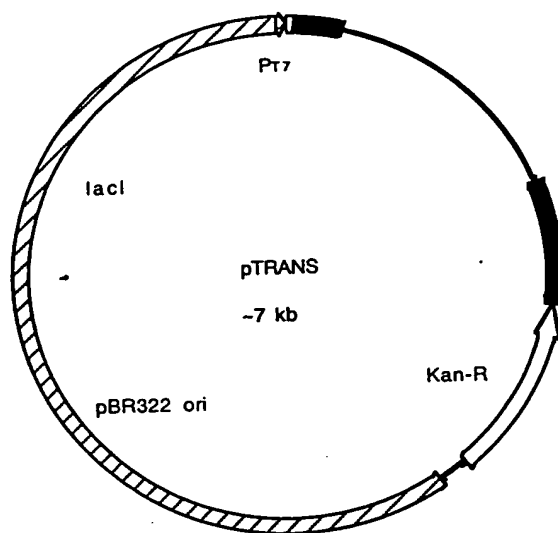
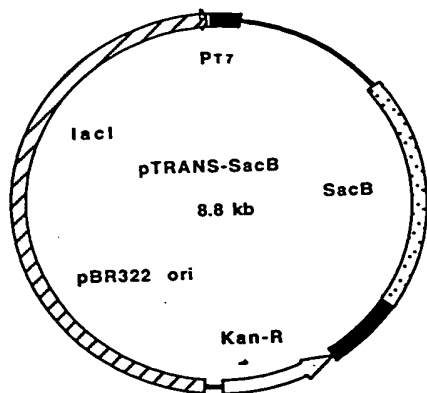


FIG 3

In vitro transposition with pTRANS-SacB (donor) and pBeloBACII (recipient)



Library BAC plasmid
(single copy #)

sucrose-R
kan-S
chlor-R

+

pTRANS-SacB
(moderate copy #)

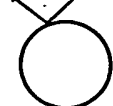
sucrose-S
kan-R
chlor-S

+

transposase

Transposase
reaction

trans-kn-ori



BACs + transposon hops
(collection of random insertions)
kan-R, sucrose-R, chlor-R



BAC
(kan-S, sucrose-R, chlor-R)

trans-kn-ori



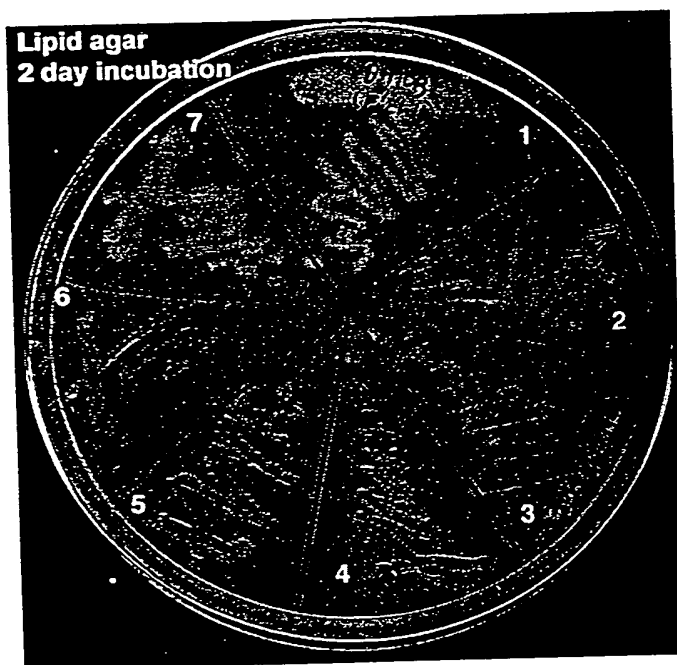
pTRANS-sacB
(sucrose-S, kan-R, chlor-S)

Transform DH10B or
DH10B(DE3) cells

select for Kan-R, chlor-R, sucrose-R transformants (on LB or specific assay medium)
Should be moderate copy # plasmids containing random transposon insertions

LOOK FOR KNOCKOUTS OF ACTIVITY AND AMPLIFICATION OF ACTIVITY

Amplification of lipase expression from a BAC clone
by increasing copy number with pTRANS-SacB



Colonies were streaked on LB agar containing
10µg/ml chloramphenicol and 3% lipid reagent
The plate was incubated at 37°C for 2 days.

#1 = non-expressing *E. coli* DH10B (negative control)

#2 = *E. coli* DH10B containing a single copy BAC plasmid
encoding a lipase gene isolated from soil DNA. Note only a
faint halo can be seen after 2 days of incubation due to
low level lipase expression.

#7 = *E. coli* DH10B containing the same BAC plasmid as in
#2, only with the lipase gene is disrupted by a *trans*
transposon. Note the plasmid in this clone is high copy,
however, the lipase activity is no longer present.

#3, #4, #5, #6 = *E. coli* DH10B containing the lipase BAC
plasmid made high-copy with a *trans* transposon outside of
the lipase gene. Note large halos surrounding clones,
resulting from digestion of lipid in the media, indicating
increased lipase expression.

FIG 5

MG1.1(parent)

Transposon hop #1
(lower expression)

Transposon hop #2
(higher expression)

pBTP2
(empty vector)

FIG 6

Fig 7

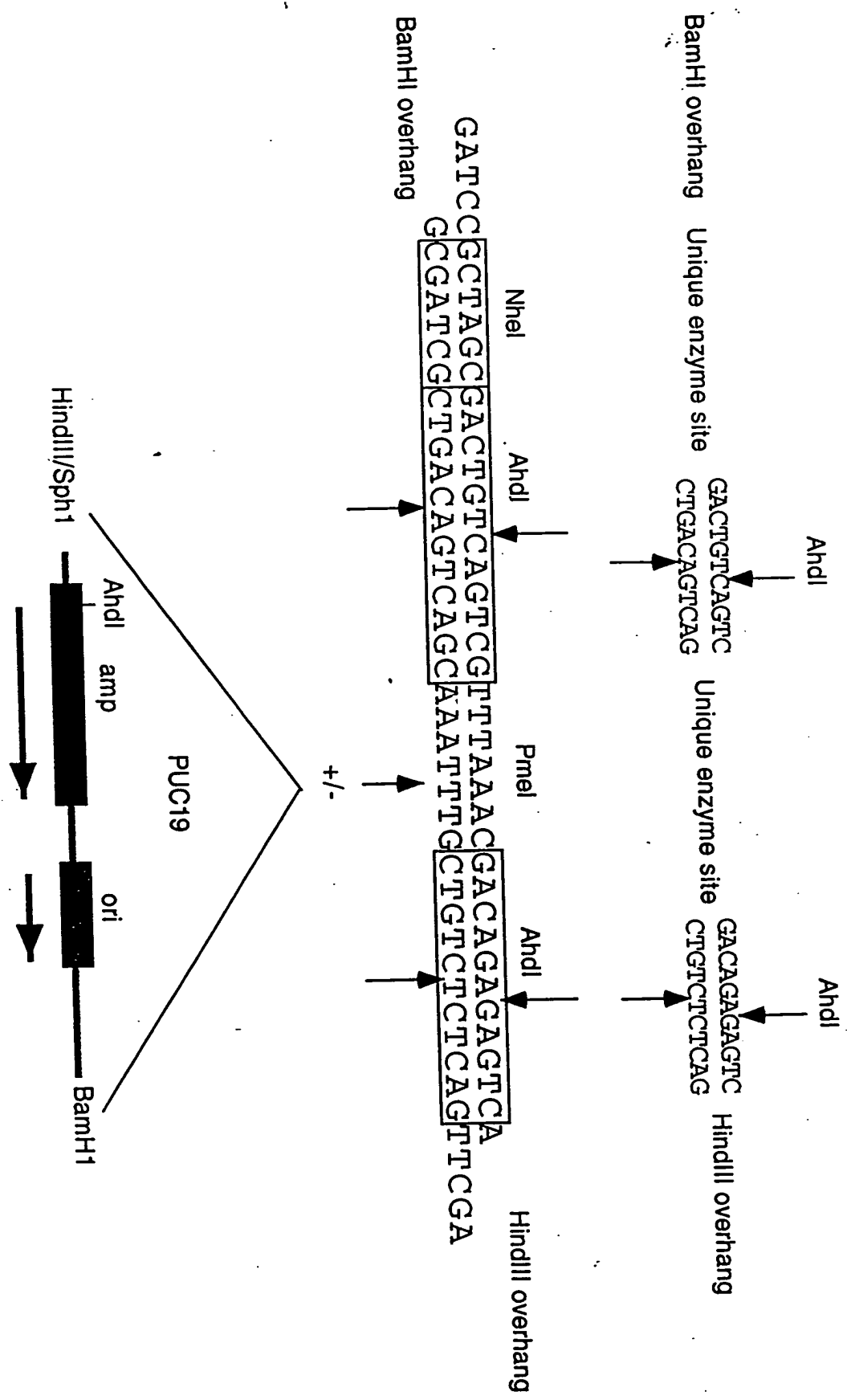


FIG 7

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SECRET

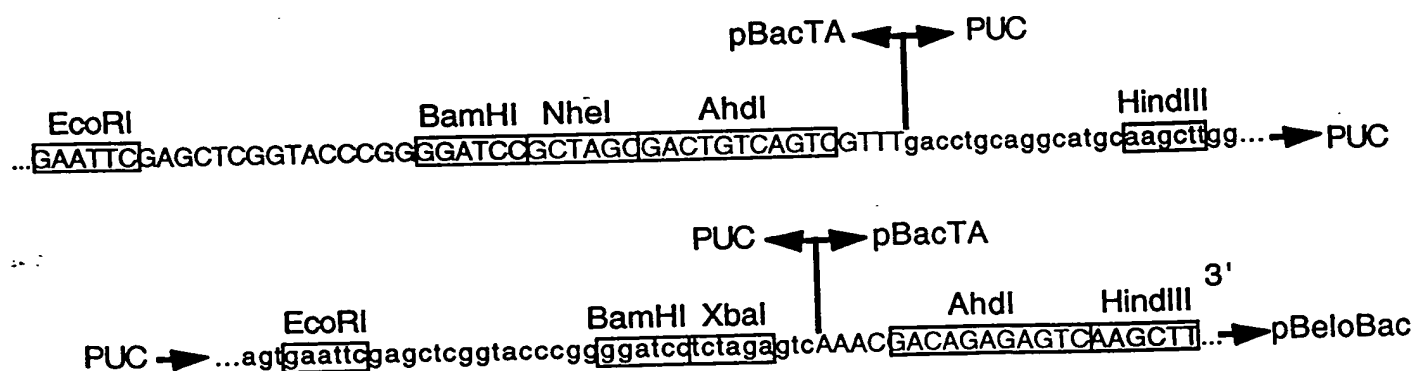


FIG 8

9

BstXI-directed Cloning

GAATTCCACCACA
CTTAAGGTG

← non-palindromic adaptor

Dirt DNA



Blunt



Linker Addition

CTGGAATTG
ACACGACCTTAAG

GAATTCCACCACA
CTTAAGGTG

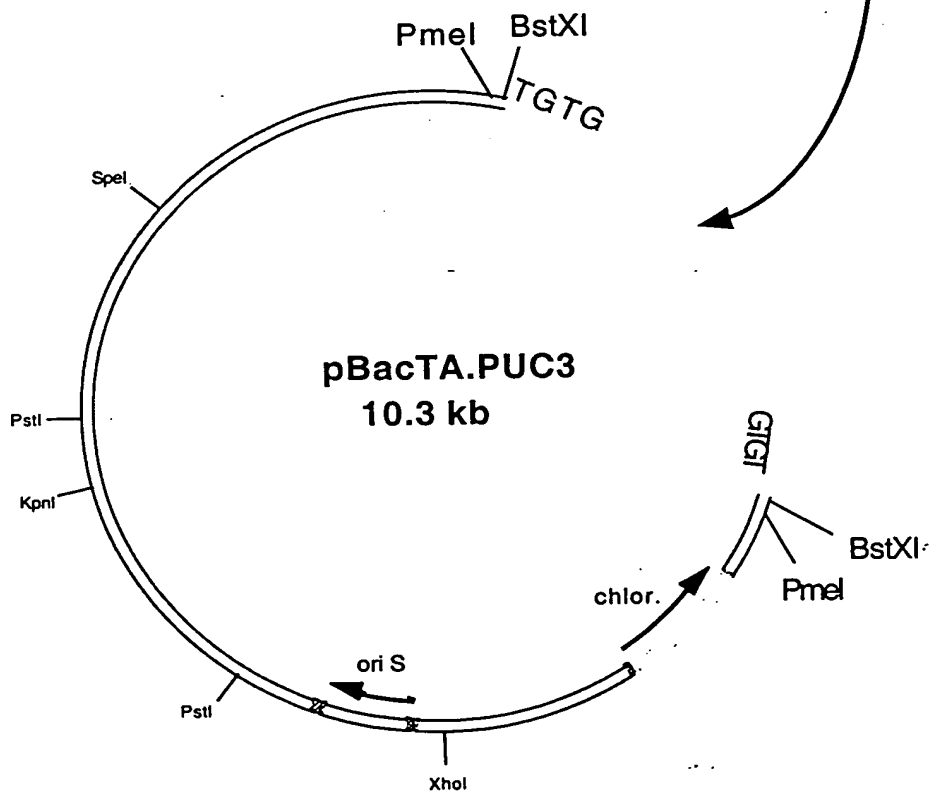
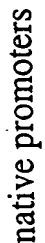


FIG 9

Multigene cluster



+

Tn-5 based promoter system

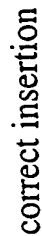


FIG 10